

Method for Full-Length cDNA Cloning Using Degenerate Stem-
Loop Annealing Primers

Abstract of the Disclosure

5 The present invention provides a method for isolating a
double-stranded cDNA having a nucleotide sequence of a
complete open reading frame which comprises: (A) admixing
(i) an isolated single-stranded cDNA, (ii) a first primer
capable of forming a stem-loop structure, comprising (a) at
10 the 3' end of the primer, a first random sequence, linked to
(b) a second sequence, linked to (c) a third sequence which
forms a loop structure, linked to (d) a fourth sequence, at
the 5' end of the first primer, which is complementary to
the second sequence, under hybridization conditions
15 sufficient for annealing the first sequence of the first
primer to the sequence at the 3' end of the single-stranded
cDNA, and (iii) a polymerase; (B) incubating the mixture
from step (A) under suitable conditions for DNA synthesis;
and (C) performing a polymerase chain reaction by admixing
20 (i) an aliquot of the mixture from (B), (ii) a second primer
which specifically binds to the single-stranded cDNA, (iii) a
third primer which comprises (a) a fifth sequence identical
to the third sequence of the first primer, linked to (b) a
sixth sequence identical to a portion of the second sequence
25 of the first primer, and (iv) a polymerase, under conditions
suitable for a polymerase chain reaction so as to produce a
double-stranded cDNA reaction product, thereby isolating the
cDNA having the sequence of the complete open reading frame.

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